CHEMISTRY.—*The use of chloroform to accelerate cyanogenesis in the analysis of cyanogenetic plants.*[1] J. F. COUCH and R. R. BRIESE, U. S. Bureau of Animal Industry. (Communicated by H. W. SCHOENING.)

In 1909 Guignard (5, 6) reported that when leaves or other tissues of cyanogenetic plants are subjected to cold or to certain anesthetics like ether or chloroform there is an increase in the rate at which hydrocyanic acid is evolved. Mirande (7) applied this discovery in modifying Guignard's picric acid paper test for the detection of cyanogenetic compounds in plants. Armstrong and his coworkers (1) developed and refined the technique applying the test to a large series of plants. Boyd and coworkers (2) used chloroform to accelerate cyanogenesis in their method for the rapid determination of HCN in Sudan grass. This technique has the disadvantage that the chloroform distills with the HCN and clouds the distillate, which obscures the end point in the subsequent titration with silver nitrate.

A rapid process for the estimation of potential HCN in cyanogenetic plants is desirable. At present none of the available methods, with the exception of the mercuric-chloride process (3), is free from large errors. Much of the inaccuracy is due to slow development of the maximum HCN, while at the same time appreciable loss of HCN occurs through its conversion into other substances (4). Loss of HCN may be prevented by the use of mercuric chloride, but this substance considerably retards the analysis by inhibiting the enzyme.

A comparative study of the rate of cyanogenesis in water with and without the addition of chloroform was made. The figures obtained by the slower mercuric-chloride method were used as controls. Sorghum varieties grown at the Arlington Experimental Farm of the U. S. Bureau of Plant Industry under controlled conditions were available through the courtesy of Dr. J. H. Martin, of that Bureau. Samples were collected at 8:30 A.M. each morning. The entire plant of young first or second growth sorghums was used, but in the case of more mature plants the stalks were discarded and the leaves only were used. Fruiting heads, when present, were discarded with the stalks.

Samples from young plants were sliced in small pieces with a sharp knife. The leaves of older plants were hashed through a meat chopper (3). In both cases the comminuted mass was thoroughly mixed and 50-g samples were weighed out for analysis as rapidly as possible. The samples for water and for chloroform treatment were weighed

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directly into 5-liter pyrex flasks and the water or water-chloroform mixture was added. One of the chloroform-water mixtures was immediately connected to a condenser and heat was applied. Fifteen minutes elapsed on the average before distillation commenced, and therefore these samples are reported in the table as being macerated one-fourth hour. Similar samples were tightly stoppered and incubated at 37° for 24 hours before analysis. Other samples were incubated with water for 24 and 48 hours at 37°. Samples for mercuric-chloride treatment were handled as previously described (3).

Twenty-four collections of seven representative sorghum varieties were used in these experiments. Nine samples were of second-growth plants, and three were of suckers. The remaining 12 samples were leaves of first-growth plants.

The results are presented in Table 1. In one case, that of second-growth Sharon kafir, the quarter-hour maceration with chloroform and the mercuric-chloride samples gave the same figure for HCN, within the limits of experimental error. In all other cases the chloroform samples gave smaller figures than the standards and in many cases so far below as to demonstrate the inadequacy of chloroform to give accurate results. In 5 cases out of 14 where the samples were macerated 24 hours the chloroform figures were lower than those for water alone. In 5 out of 13 cases they were lower than the figures for 48-hour water maceration. It appears that chloroform applied in this way can not be depended upon to give accurate as well as rapid results.

A few experiments were performed to test the utility of Roe's method for amygdalin (8) when applied directly to the plant. Samples weighing 25 g were mixed with 1 cc of chloroform, placed in a small desiccator arranged so that air might be drawn through the mass and thence conducted through a solution of potassium hydroxide to catch the entrained HCN. Before entering the desiccator the air was drawn through a scrubber containing potassium hydroxide. After several hours the cyanide trapped in the absorption apparatus was titrated according to Liebig-Denigès. A new absorption train was connected to the desiccator and air was drawn through long enough to make the total aeration period 10 to 24 hours. The results are presented in Table 2 and are compared with the results obtained after maceration of another sample in water at 37° for 24 hours. The standard is the figure obtained by the mercuric-chloride process. In one case only, that of Sharon kafir, the chloroform sample gave a figure in excess of the water-macerated sample. In all instances the results of the

TABLE 1.—HYDROCYANIC ACID RECOVERED FROM SORGHUMS AFTER MACERATION IN WATER WITH AND WITHOUT THE ADDITION OF CHLOROFORM COMPARED WITH A STANDARD BASED ON PRESERVATION IN MERCURIC CHLORIDE

Date		Variety	Stage of growth	Part of plant	Height	How prepared	Moisture	Hydrocyanic acid per 100 g calculated to dry plant				
								Standard	After maceration in—			
									Water for—		Water and chloroform for—	
									24 hrs.	48 hrs.	15 min.	24 hrs.
1939					*inches*		*Percent*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
July	17	Hegari	First	Leaves	24	Ground	79.76	208	140	188	145	...
	18	Sumac sorgo	Suckers	"	10–14	Sliced	84.03	189	138	139	111	...
	19	Sharon kafir	First	"	24–26	Ground	82.54	85	61	50	52	...
	20	Spur feterita	Suckers	"	20–22	"	85.15	273	256	256	216	...
	24	Hegari	First	"	24–26	"	81.96	284	139	151	176	...
	25	Sumac sorgo	"	"	36	"	81.79	184	131	141	114	...
	26	Sharon kafir	"	"	38–40	"	79.73	89	76	64	66	...
	28	Atlas sorgo	"	"	48	"	82.70	88	72	...	50	...
	31	Hegari	"	"	48	"	78.21	246	207	199	150	158
Aug.	1	Hegari	Suckers	"	18	"	83.90	257	160	228	182	210
	2	Sumac sorgo	First	"	72	"	80.12	152	119	116	...	89
	3	Spur feterita	"	"	54	"	78.71	256	210	201	169	201
	4	Dwarf Yellow milo	"	"	36	"	79.21	144	115	...	87	101
	7	Hegari	"	"	60	"	78.57	225	184	194	148	172
	8	Hegari	Second	Whole	10–12	Sliced	89.29	405	324	304	308	345
	8	Sharon kafir	"	"	10–12	"	88.42	196	139	136	198	156
	15	Ajax	"	"	6–8	"	88.16	583	444	502	481	563
	22	Hegari	"	"	10–12	"	87.00	325	316	302	251	...
	29	Dwarf Yellow milo	"	"	12–14	"	92.55	318	227	224	170	...
September	26	Spur feterita	"	"	6–10	"	87.68	238	217	198	188	219
October	10	Hegari	"	Leaves	54–60	Ground	72.97	114	47	91	54	65
	11	Ajax	"	Whole	8–12	Sliced	83.44	228	163	152	133	196
	16	Hegari[1]	First	Leaves	60–72	Ground	71.00	75	53	50	22	63
	17	Hegari[1]	Second	Whole	8–12	Sliced	84.86	297	198	208	176	273

[1] Frosted.

aeration process were much below the standard. Even with a sample of wild-cherry leaves (*Prunus serotina*), a species in which cyanogenesis is much more rapid than in sorghum, the aeration process yielded less than 50 percent of the standard. Adding small quantities of water to the plant mixtures did not improve the evolution sufficiently to make the process suitable as an accurate analytical procedure.

The reason for the failure of the aeration method lies in the slowness of cyanogenesis under the experimental conditions. Roe obtained excellent results with amygdalin and emulsin in solution, a condition in which the glucoside and enzyme may readily come into contact. In hashed plant tissues, even where the permeability of the cell wall is increased by chloroform, it is more difficult to bring the glucoside

TABLE 2.—HYDROCYANIC ACID RECOVERED FROM CYANOGENETIC PLANTS BY THE AERATION PROCESS

Date	Variety	Height	Moisture	Hydrocyanic acid per 100 g calculated to dry plant			Remarks
				After maceration in water for 24 hours	After aeration with $CHCl_3$	Standard	
1938		*inches*	*Percent*	*Mg.*	*Mg.*	*Mg.*	
September 26	Hegari	72	72.71	26	19	32	Leaves
28	Sharon kafir	12–14	86.41	76	82	110	Second growth
October 3	*Prunus serotina*		57.75	454	259	555	Leaves
17	Spur feterita	10–12	85.03	154	67	208	Second growth
5	Spur feterita	8–14	81.33	164	46	210	"

and enzyme into contact. The addition of 10 to 20 parts of water and maceration at 37° for 24 hours seldom develop the maximum quantity of HCN potential in the plant. This is due to a combination of factors, but adsorption of the enzyme on the plant fiber and consequent inhibition of contact with the glucoside appear to play an important rôle in this respect.

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